(51) International Patent Classification 4 :	(11) International Publication Number:	WO 86/ 06487
G01N 33/53, 33/68	**A1**	
	(43) International Publication Date: 6 November 1986 (06.11.86)	

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(54) Title: METHOD FOR DETERMINING MIMOTOPES

(57) Abstract

A method of detecting or determining the sequence of monomers which is a topographical equivalent of the ligand which is complementary to a particular receptor of interest, the method comprises the steps of: 1) synthesizing a plurality of catamers, each said catamer being of the general formula: D_2-D_1, wherein D_1 represents a designated monomer selected from a first set of monomers, and D_2 represents a designated monomer selected from a second set of monomers which may be the same as or different to said first set of monomers; said plurality of catamers comprising catamers in which each designated monomer is systematically varied to contain members from the respective set of monomers; 2) contacting each catamer with the receptor of interest, and, 3) detecting or determining the presence or absence of binding between each catamer and said receptor. The method may also include the synthesis of further pluralities of catamers, including catamers containing spacer molecules, to build up a sequence of monomers which is the mimotope of the ligand.

"IMPROVED METHOD FOR DETERMINING MIMOTOPES"

This invention relates to a method of
detecting or determining a sequence of monomer molecules
which corresponds to the ligand molecule for a
particular receptor. The sequence of monomer molecules
5   so determined is the mimotope (defined below) of the
particular ligand. The mimotope which is determined by
this method may not have any obvious or direct
relationship to the natural ligand molecule, but will
share with it the ability to react with the receptor,
10   and indeed, the mimotope so determined may be modified
to incorporate specific or additional properties in the
reaction with the receptor. Such a mimotope could then
be used to replace the natural ligand in the treatment
or prevention of particular disease or it may be used to
15   mediate a particular biological effect.

As used throughout this specification, the
terms listed below have the following meanings:-

20   receptor: a molecule or molecular complex which
                    will combine specifically with its particular
                    ligand molecule. It is those receptors which
                    on binding with their particular ligand(s)
                    mediate a biological function that are of most
25   interest. Examples of receptors include, but

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are not restricted to, the common class of
receptors associated with the surface membrane
of cells and include, for instance, the
immunologically important receptors of

5   B-cells, T-cells, macrophages and the like.
Another example is receptors for acetyl
choline on nerve cells which cause a nerve
pulse to be transmitted down the length of the
neuron when the receptor molecule reacts with

10   its ligand, acetyl choline.

epitope: the specific surface of an antigen
molecule which is delineated by the area of
interaction with the sub-class of receptors

15   known as antibodies.

catamer: a polymer molecule which is a precisely
defined linear sequence formed by the
condensation of small molecules. Note that

20   this term includes molecules in which
different types of condensation reactions are
used to join the small molecules. A number
prefixed to the word "catamer" implies that
the catamer is formed by the condensation of

25   the indicated number of small molecules, for
example, 8-catamer means that the catamer is
made up from eight small molecules. Examples
of catamers include any given peptide and any
given oligo-saccharide.

30

monomer: a member of the set of small molecules
which can be condensed together to form a
catamer. The set of monomers includes but is
not restricted to, for example, the set of

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common L-amino acids, the set of D-amino
acids, the set of synthetic amino acids, the
set of nucleotides, and the set of pentoses
and hexoses.

5

peptide: a catamer in which the small molecules
are alpha-amino acids and which are joined
together through a peptide bond. In the
context of this specification it should be

10   appreciated that the amino acids may be the
L-optical isomer or the D-optical isomer.

mimotope: a catamer which in at least one of its
conformations has a surface region with the

15   equivalent molecular topology to the binding
surface of the ligand molecule of which it is
the mimic. In the context of immunological
receptors, the mimotope mimics the epitope of
the antigen.

20

complementary: refers to the matching together of
the reacting surfaces of an ligand molecule
and its receptor. Thus, the receptor and its
ligand can be described as complementary, and

25   furthermore, the contact surface
characteristics are complementary to each
other.

paratope: the combining site of an antibody which

30   is complementary to a particular epitope.

ligand molecule: is the molecule which binds to
a particular receptor and when bound to it

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mediates the biological function associated with that particular receptor.

Examples of receptors which can be
5   investigated by this method include, but are not restricted to:-

hormone receptors: for instance the receptors for insulin and Growth Hormone; determination of
10   the mimotopes of the ligands binding to these receptors may lead to the development of an oral replacement of the daily injections which diabetics must take to relieve the symptoms of diabetes, and in the other case, a replacement
15   for the scarce human Growth Hormone which can only be obtained from cadavers or by recombinant DNA technology. Other examples are the vasoconstrictive hormone receptors; determination of the mimotope of the ligand
20   binding to these receptors may lead to the development of drugs to control blood pressure.

opiate receptors: determination of mimotopes of
25   the ligands binding to the opiate-receptors in the brain may lead to the development of less-addictive replacements for morphine and related drugs.

30   microorganism receptors: determination of mimotopes of the ligands binding to a receptor such as specific transport proteins or enzymes essential to survival to microorganisms, may lead to a new class of antibiotics. Of
35   particular value would be antibiotics against

protozoa and those bacteria resistant to the
antibiotics in current use.

enzymes: for instance, the enzymes responsible for
cleaving neural transmitters; determination
of mimotopes able to modulate the action of
the enzymes which cleave the different neural
transmitters may lead to the development of
drugs which can be used in the treatment of
disorders of neural transmission; and,

antibodies: for instance the ligand-binding site
on the antibody molecule which combines with
the epitope of an antigen of interest;
determination of a mimotope for the epitope
may lead to the development of vaccines of
which the immunogen is based on one or more
mimotopes or diagnostic agents or compounds
useful in the therapeutic treatment of
autoimmune diseases.

Examples of ligands which can be investigated
by this method include, but are not restricted to:-

toxins and venoms: for instance, the combining
site of the toxin molecule which reacts with a
particular receptor in the body to give the
particular symptom(s) of intoxication;
determination of mimotopes to the combining
site of the ligand may lead to the development
of drugs which can be used to treat
envenomation by snakes and other poisonous
animals without the side effects of
heterologous antivenenes.

virus and other microorganism capsid molecules:

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for instance, the combining site on the virus
coat molecule which reacts with a particular
receptor on the cell membrane in the body and
which allows the virus to invade and thus

5   infect the particular cell; determination of
mimotopes to this combining site may lead to
the development of drugs which specifically
prevent intracellular invasion by the virus
and thus prevent their replication.

10
It is a primary object of this invention to
detect or determine one or more short sequences of
monomers (catamers) which selectively combine with a
particular receptor so as to mediate its biological

15   function. These catamers are the mimotopes of the
ligand. This information is invaluable for the design
of very specific diagnostic and therapeutic agents.

The most usual group of small molecules which
20   may be condensed together to form a catamer is the group
of alpha-amino acids. However, other molecules which
are consistent with a different chosen chemistry may
also be used; for example, catamers formed from the
specified sequential condensation of nucleotides or

25   saccharides. Another group of small molecules would be
the non-genetically coded amino acids such as beta-amino
acids, which may be used to advantage to add an
additional bond at specified positions within the
catamer.

30
The method of the present invention is based
on the realisation that a given receptor will
specifically react with a catamer which is the mimotope
for the ligand to which the receptor is directed. It

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further relies on modern techniques of immunology to
detect reaction between a receptor and its ligand when
both are present.

5   In Australian Patent Specification No.
45339/85 it is proposed to delineate mimotopes based on
an overall length of about 8 monomers. It is now clear
that the preferred method for the delineation of
mimotopes is from shorter catamers (2 or 3 monomers
10   long) made up from all combinations of monomers from
either:-

(i)   two sets of monomers, which may be identical;   or
(ii)   three sets of monomers, of which the centre monomer
       of the catamer is selected from a set of special
15   chosen monomers which confer known spatial
       relationships on the other two monomers, the
       remaining monomers of the catamer coming from two
       sets of monomers which may be identical.

Furthermore, it has now been shown that the sensitivity
20   of detection of binding to receptors is reduced in some
instances where the monomers are synthesized in catamer
preparations as disclosed in Patent Specification No.
45339/85.

25   We have now demonstrated that reaction is
readily detected between a receptor and short mimotopes.
These short mimotopes when condensed together then bind
to the receptor with either a greater affinity or with
greater specificity for that receptor. This reaction
30   can be detected even when the short mimotope presented
to the receptor is as small as two monomer molecules
long. By determining the optimum short mimotope at each
stage and then testing further variants, the final
structure of a strongly-binding mimotope can be
35   determined.

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According to the present invention there is provided a method of detecting or determining the sequence of monomers which is a topographical equivalent of the ligand which is complementary to the particular receptor of interest and prior knowledge is irrelevant about the identity, structure and sequence of the receptor or its ligand. The method comprises the steps of:-

1. synthesizing a plurality of catamers, each said catamer being of the general formula:

$$D_2 - D_1$$

wherein D_1 represents a designated monomer selected from a first set of monomers, and D_2 represents a designated monomer selected from a second set of monomers which may be the same as or different to said first set of monomers; said plurality of catamers comprising catamers in which each designated monomer is systematically varied to contain members from the respective set of monomers;

2. contacting each catamer with the receptor of interest, and,

3. detecting or determining the presence or absence of binding between each catamer and said receptor.

The method may also comprise the further steps of:

a. synthesizing a further plurality of catamers, each said catamer being of the general formula:

$$D_3 - D_2 - D_1, \text{ or}$$

$$D_2-D_1-D_3$$
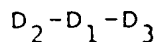
wherein D_1 and D_2 are as defined above and preferably represent a combination of monomers corresponding to a catamer which binds to said receptor, and D_3 represents a designated monomer selected from a third set of monomers which may be the same as or different to either the first or the second set of monomers; and

b. performing steps 2 and 3 as described with the further plurality of catamers.

The procedure of steps a. and b. above may be repeated to further "extend" the catamers by systematically adding further monomers to the catamers, and testing in the same manner as in step b, above.

In another important aspect, the method of this invention may comprise the steps of:

A. synthesizing a plurality of additional catamers, each said additional catamer being of the general formula:

$$D_2-Sp-D_1$$
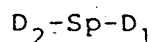
wherein D_1 and D_2 are as defined above and Sp is a spacer molecule which can modify the relative orientation of the monomers D_1 and D_2; and

B. performing steps 2 and 3 as described above with the plurality of additional catamers.

The spacer molecule "Sp" as described above may also be systematically introduced into all possible positions of

the "extended" catamers referred to above, and tested in the same manner as in Step B. above.

In yet another aspect, the method of the invention may further comprise the steps of systematically replacing the monomers in any of the catamers referred to above with their optical isomers, either individually or in combinations of monomers, and again performing steps 2 and 3 as described above.

In a further aspect, the method may further comprise the steps of systematically replacing the monomers in any of the catamers referred to above which bind with the receptor of interest, either individually or in combinations of monomers, with other monomers selected from the respective set(s) of monomers, and again performing steps 2 and 3 as described above.

It will be apparent that the method of this invention requires no previous information about the nature of the ligand and in particular it requires no foreknowledge of the sequence of monomers which make up the ligand. In fact, it is not necessary for the application of this invention to know the source or identity of the ligand to which the receptor is directed. Furthermore, this invention makes no assumptions about the nature of the ligand of the particular receptor. This method will identify mimotopes of discontinuous as well as continuous ligands. Because of the very nature of the method of the invention it will be appreciated that mimotopes may or may not consist of members of the same set of monomers which make up the ligand of which it is the mimic.

The plurality of catamers required to be synthesized in the application of this invention may be prepared by any of the known method of catamer synthesis. The preferred methods when the monomers are amino acids is to use the solid phase technique described in Australian Patent Specification No. 25429/84, whereby each catamer is synthesized on a polyethylene support.

The following is a detailed description of one embodiment of the present invention when applied to the determination of a mimotope for a ligand able to bind to a receptor when that receptor is an antibody. In this context, the ligand is usually referred to as the epitope for the antibody. Preferably the method of the present invention is carried out by screening a plurality of synthesized catamers against the antibody of interest. Ideally the antibody will be a monoclonal antibody which can be prepared by any of the usual methods. Polyclonal antiserum from humans and animals may be used if a source of monoclonal antibody with the desired characteristics is not available, however, analysis of the resulting data may be complicated because the reaction observed could be from more than one monoclonal antibody. When using polyclonal antiserum it may be necessary to reduce the antibody diversity by using techniques well known to those skilled in the art, for example iso-electric focusing, HPLC-based chromatography or affinity chromatography.

Current indications suggest that an epitope mimicked by a catamer which is usually about six monomers in length when the monomers come from the set of alpha-amino acids. It is to be understood, however,

that the present invention is not restricted to
sequences formed from six monomers. The ability of the
6-catamer to be the mimotope of the epitope is not
critically dependent on every position having a

5   designated monomer. It has been found that certain
positions in most mimotopes are not restricted to a
single designated monomer for binding with the receptor.

A.   Synthesis of a plurality of catamers.

10   As noted above, the preferred method of
applying this invention is to synthesize the catamers on
a solid support. In this embodiment, the plurality of
catamers will all have the general formula:

15   $$Y-D_2-D_1-Lk-(solid\ support),$$
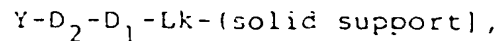
where "Lk" represents a linker molecule which provides a
suitable end group for condensing the monomers to the
solid support. "D_1" and "D_2" represent designated
positions occupied by monomers which are selected from

20   known sets of monomers; but which are altered
systematically between catamers. It should be noted
that the set of monomers used for the D_1 designated
position need not be the same set of monomers used for
the D_2 designated position. "Y" in the general formula

25   is an end group of the catamer and may be, but is not
restricted to, for example a hydrogen atom or an acetyl
group. "Y" may also be another molecule which is
coupled to the catamer to preserve particular

30   characteristics of the molecular environment of a
peptide bond at the amino terminal designated position.

If i is the number of members in the set of
monomers to be coupled in the D_1 position and j is the

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number of members in the set of monomers to be coupled in the D_2 position then a total of i. j different catamers will be synthesized.

5   In the present embodiment, the support rods are prepared so that the monomers can be coupled to them by coupling an appropriate linker molecule.

      For the coupling at the D_1 position, each rod
10   will be treated with a reaction mixture which contains only a single monomer such as a protected amino acid or the like. In this position each of the i monomers are coupled to j rods. For the coupling at the D_2 position each rod is treated with a reaction mixture which
15   contains a single monomer such as a protected amino acid or the like. Each of the j rods which has a particular monomer in the D_1 position will have a different monomer coupled at the D_2 position. In this way every combination of the members of the set(s) of monomers
20   will be found in the i. j rods.

      The desired end group, "Y", is then coupled using the appropriate chemistry.

25   After synthesis of the plurality of catamers any side-chain protective groups are removed from the catamers using the appropriate techniques and the rod-coupled catamers are washed.

30   It has been found to be a preferred embodiment of the invention to synthesize more than one set of plurality of catamers to aid in the analysis of data. Thus, as well as synthesizing catamers with the general formula

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$$Y-D_2-D_1-Lk-(solid\ support)$$
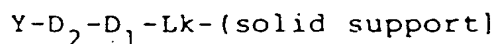
as described above, additional sets of catamers may be prepared with the general formula

$$Y-D_2-Sp-D_1-Lk-(solid\ support)$$
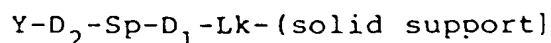
where "Sp" is a spacer molecule which may restrict the relative orientation of the monomers at the designated positions to a particular geometrical configuration(s). The spacer molecule may also be deliberately chosen to allow a greater flexibility to the relative geometric configuration between monomers in the designated positions, D_1 and D_2. It should be noted that members of the set of spacer molecules may be made up from the condensation of more than one monomer. Examples of spacer molecules include, but are not restricted to glycine (approximately linear extension), beta-alanine (increased flexibility), proline (forced bend), glycyl-proline (extended bend, otherwise known as reverse bend in protein structure terminology) and o-aminobenzoic acid (a planar bend).

By analyzing the results from these sets of catamers the preferred spatial relationships between monomers in the mimotopes can be deduced as will be shown in the appropriate examples given below.

B.   Testing of the plurality of catamers.

The plurality of catamers prepared as in A. above are then contacted with the particular antibody of interest. The reaction between antibody and each catamer can then be detected by any of the usual methods, for example, radioimmunoassay (RIA). However,

the preferred method of detection is to use the well known enzyme-linked immunosorbent assay (ELISA).

5   At the end of each assay antibodies can be removed from the catamers by, for example, washing with a solution of 8M urea, 0.1% 2-mercaptoethanol and 0.1% sodium dodecylsulphate followed by several washes in phosphate buffered saline. In this way the plurality of catamers may be used for testing with many other

10   antibodies.

C.   Analyses of the data

      In the testing of a set of catamers with antibody it has been found that certain catamers will

15   show detectable binding with the antibody. These reacting catamers identify useful combinations of the members of the set(s) of monomers. These combinations of monomers are short mimotopes which, when extended, may bind to the antibody with a greater affinity or

20   alters specificity. Analysis of the data is greatly facilitated by including a number of control peptides in the synthesis which aid the determination of significant responses. Further analysis of the data can be carried out in a number of ways. These include:-

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1.   permutating each of these reacting combinations of monomers to create a list of catamers which will include mimotopes which bind to the paratope; each catamer in this list can be regarded as a possible

30   mimotope;

2.   selecting candidates from the list of reacting combinations of monomers and further synthesizing sets of catamers in which the known reacting

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combination of monomers is held constant and
further monomers are added systematically at either
end; thus in the example above, a suitable set of
such catamers would include catamers with the
formulae

$$Y-D_3-A_2-A_1-Lk-(solid\ support)$$

and

$$Y-A_2-A_1-D_3-Lk-(solid\ support)$$
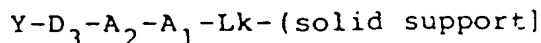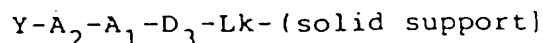
where A_1 and A_2 constitute the reacting combination
of monomers from the previous results and D_3 is the
new designated position where each of the members
of the set of monomers is systematically varied;
and

3. combining the results from different sets of a
plurality of catamers and analyzing the results to
deduce a single sequence of monomers, or a small
number of such sequences which would bind to the
antibody of interest. Analysis of this data is
possible as the reacting combinations of monomers
when they are adjacent to each other are now known,
as well as the reacting combinations of monomers
when they have particular geometrical
configurations between them. In this way, these
data can then be interpreted to predict the
structure of the mimotope when bound to the
antibody; this approach will be of greatest
benefit when the antibody used to test the
plurality of catamers is a monoclonal antibody.

The procedure given in 2. above can be
repeated until no further enhancement of binding is
achieved by additional extending of the mimotope.
Ideally, the sequence of the mimotope should be checked

by regularly synthesizing and testing replacement nets
of the mimotope to obtain the optimally-binding mimotope
for further extension as described in Australian Patent
Specification No. 25428/84.

5

D.   Synthesis of selected catamers

The selected catamers can be synthesized using
similar methods to those used in A. After the selected
catamers have been synthesized they are reacted with the
10   antibody of interest. It is then simple to select the
catamer which binds most strongly with the antibody.

The binding of the mimotope with the antibody
can be further enhanced by synthesizing a further
15   plurality of catamers based on the sequence of the most
strongly binding mimotope. This plurality of catamers
consists of adding spacer molecules (-Sp-)
systematically at all positions of the mimotope; and
where feasible systematically replacing each monomer of
20   the catamer with its optical isomer. Testing of this
set of catamers with the antibody will give invaluable
information about the relative orientation of the
monomers and their stereochemistry as required for
binding with the antibody.

25

In Examples 1, 2, 3 and 4 given below, the
mimotopes are being determined for the antigen against
which different monoclonal antibodies were raised. The
defined set of monomers is the set of the common L-alpha
30   acids unless otherwise specified. The linker molecule,
-Lk-, was 3 amino-N[1] (6-aminohexyl)-propanamide and the
end group, Y-, was the acetyl moiety. In Example 5
below, the mimotope is determined for an antigenic
determinant of human chorionic gonadotrophin which

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induced a monoclonal antibody. The linker molecule, -Lk-, is the same as that used in earlier Examples. The end group, Y-, is either ß-alanyl-ß-alanine or ß-alanine. The defined set of monomers was extended to

5   include the D-optical isomers of the common alpha amino acids and several unusual amino acids. In Example 6 below, the mimotope is determined for a receptor site on viruses. The linker molecule, -Lk-, is the same as for earlier Examples. The end group, Y-, is either

10   ß-alanyl-ß-alanine or ß-alanine. The defined set of monomers was extended as described for Example 5.

EXAMPLE 1

    A monoclonal antibody was raised against sperm

15   whale myoglobin using the usual techniques. This monoclonal antibody was tested against a set of catamers with the general formula:

$$Y-D_2-D_1-Lk-(solid\ support).$$
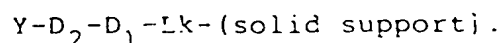
20   Three pairs of reacting monomers bound to the catamers with approximately equal response and were significantly higher than other pairs of monomers. The sequences were E-F, E-L and E-H.

25   A further set of catamers was synthesized which comprised all 4-catamers which could be made from the set of monomers; Glutamic acid (E), Phenylalanine (F), Histidine (H) and Leucine (L). The following catamers were found to bind significantly higher than the

30   remaining catamers:-

        L-H-E-F

        L-H-F-E

        F-L-H-E

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F-H-L-E

H-E-F-L

This small group of short mimotopes can now become the
candidates for further extensions.

EXAMPLE 2

A monoclonal antibody was raised against sperm
whale myoglobin using the usual techniques. This
monoclonal antibody was tested against a set of catamers
with the general formula:

$Y-D_2-D_1-Lk-$(solid support).

Three pairs of reacting monomers bound to the catamers
with approximately equal response and were significantly
higher than other pairs of monomers. The sequences were
E-F, E-L and E-H.

Six further sets of catamers were synthesized using
the pairs E-F, E-L and E-H as starting points. Using
the E-F pair as an example. the catamers in each set had
the general formula: -

$Y-E-F-Lk-$(solid support), or

$Y-E-Sp-F-Lk-$(solid support),

where Sp is a spacer molecule from the set of
beta-alanine, glycine and L-proline. Furthermore the
D-optical isomer of both the Glutamic acid and
Phenylalanine were systematically substituted for the
L-optical isomer.

The catamers which gave the best response from each
set of catamers were

D-Glutamic acid - L-Proline - L-Phenylalanine

L-Glutamic acid - L-Leucine, and

D-Glutamic acid - L-Proline - D-Histidine.

These results show that the monomers F and H were better
positioned non-adjacent to E; furthermore, E and L are
best positioned adjacent. The optical isomers in the
catamers which gave the best binding suggest a structure
for a strongly binding mimotope as set out below. It is
to be noted that the sperm whale myoglobin molecule does
have a region which is similar to the predicted
mimotope. Obviously, this predicted mimotope becomes a
candidate for further extension.

The structure below is a two dimensional
representation of the spatial relationship between the
amino acids, F, E, L, and H when bound to a monoclonal
antibody against sperm whale myoglobin (see Example 2).
It must be noted that this is an illustration only and
must not be interpreted to mean that the catamer
illustrated is planar. Furthermore, the bonds joining F
to E and L to H are meant to represent a distance
greater than that of a peptide bond.

```
       ⸍F
       |
      ⸏E - L⸜
            ⎤
          H⸌
```
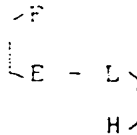
When this structure is compared with the X-ray
crystallography structure of myoglobin, it is of
considerable interest to note that the sequence
-F-L-E-L- appears at positions 135 to 138 and that there
are two histidine residues (at positions 81 and 82) in
close proximity to the glutamic acid at position 136.
This gives considerable credence to the postulated
structure of the epitope of the monoclonal antibody.

EXAMPLE 3

A mimotope to a monoclonal antiserum raised against
Foot and Mouth Disease Virus was delineated to the
sequence W-Q-M-G-H-S. A series of catamers were

synthesized in which a beta-alanine residue was
introduced systematically between monomers. The
sequence W-Q-M-β-G-H-S gave a response which was
significantly larger than the base sequence, where β
represents beta-alanine. It was further found that
excellent binding to the antibody was achieved with the
sequences:

$$W-Q-M-\beta-\beta-H-S$$

$$W-Q-M-\beta-\beta-\beta-H-S$$

which suggests that the glycine in the starting mimotope
was a spacer between two reacting elements, W-Q-M and
H-S. Thus it can be clearly seen that the mimotope is
made up of two parts; furthermore, the joining together
of these parts is not critical for the mimotope to be
able to react strongly with the antibody.

A further set of catamers were synthesized in which
the D-optical isomer systematically replaced the
L-optical isomer monomer in the starting mimotope. The
results of testing with the antibody showed that for
strongest binding, the monomers in each element should
have the same optical isomer and that there was a
preference for the monomers in the W-Q-M element to be
the D-optical isomers whereas the monomers in element
H-S should be L-optical isomers. These results can be
interpreted to mean that the epitope to the antibody is
made up of two adjacent anti-parallel chains in which
the chain direction is M-Q-W and the second chain is
H-S. This prediction leads to the suggestion that
another mimotope to the monoclonal antibody would be:-

$$G-H-S-\beta-G-W-Q-M$$

This was synthesized and found to react with comparable
binding to the antibody as the strongly binding
catamer:-

W-Q-M-β-G-H-S.

Thus, the way in which the two elements W-Q-M and H-S are joined together is irrelevant to the ability to combine with the antibody so long as their relative positions remain the same. The best mimotope as a potential immunogen will be one in which these elements are joined together from both sides in order to minimize conformational mobility.

EXAMPLE 4

A monoclonal antibody (SO93-7) raised against Foot and Mouth Disease virus was tested with catamers with the general formula:-

$$Y-D_2-D_1-Lk-(solid\ support)$$
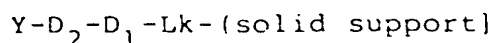
It was found that the dipeptide Q-F reacted significantly more strongly with the monoclonal antibody than with any other dipeptide.

Further sets of catamers were synthesized with the general formulae:

$$Y-Q-F-D_3-Lk-(solid\ support)$$

and

$$Y-D_3-Q-F-Lk-(solid\ support)$$
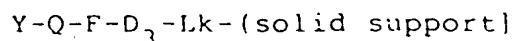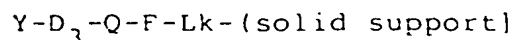
When these sets of catamers were reacted with the antiserum it was found that the following catamers reacted significantly better with the monoclonal antibody than any of the others:-

H-Q-F

N-Q-F

G-Q-F

Q-F-Q

Q-F-G

This small group of short mimotopes can now become the candidates for further extensions.

EXAMPLE 5

A monoclonal antibody (S218-4) was raised against human chorionic gonadotrophin using the usual techniques. This was tested with catamers with the general formula:-

$$Y_1-D_2-D_1-Lk-(solid\ support)$$
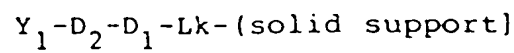
where Y_1- is ß-alanyl-ß-alanine. It was found that the dipeptide in the designated positions which bound most strongly to the monoclonal antibody was F-A.

Further sets of catamers were synthesized with the general formulae:-

$$Y_2-D_3-Sp-F-A-Lk-(solid\ support)$$

and

$$Y_1-F-A-Sp-D_3-Lk-(solid\ support)$$
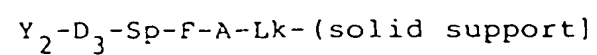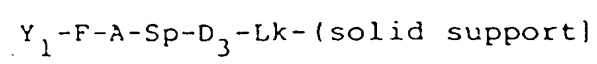
where Sp is a spacer which is an element of the set (null, ß-alanine), and Y_2- is the end group, ß-alanyl. The set of monomers used in the designated position D_3 was extended to include both the L- and D- optical isomers of the common alpha amino acids, and the unusual amino acids, α-amino butyric acid, γ-amino butyric acid, L-norcleucine, sarcosine, ornithine, L-norvaline, L-homophenylalanine, 3-alanine. When these sets of catamers were reacted with the monoclonal antibody it was found that the catamers which reacted strongly were:-

$$Y_2-P_D-F-A$$

and

$$Y_2-A_D-F-A$$
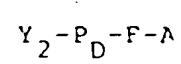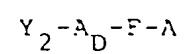
where P_D and A_D represent the D-optical isomers of proline and alanine, respectively.

Further sets of catamers were synthesized with the general formulae:-

$$Y_2\text{-}D_4\text{-}Sp\text{-}P_D\text{-}F\text{-}A\text{-}Lk\text{-}(solid\ support)$$
$$Y_1\text{-}P_D\text{-}F\text{-}A\text{-}Sp\text{-}D_4\text{-}Lk\text{-}(solid\ support)$$
$$Y_2\text{-}D_4\text{-}Sp\text{-}A_D\text{-}F\text{-}A\text{-}Lk\text{-}(solid\ support)$$

and
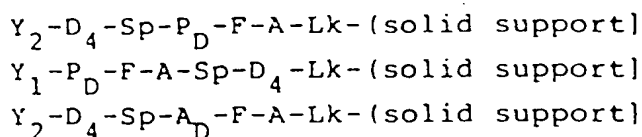
$$Y_1\text{-}A_D\text{-}F\text{-}A\text{-}Sp\text{-}D_4\text{-}Lk\text{-}(solid\ support)$$

where the extended set of monomers was used in the designated position D_4. When these sets of catamers were reacted with the monoclonal antibody it was found that the catamer which reacted most strongly was:-

$$Y_1\text{-}P_D\text{-}F\text{-}A\text{-}D_D$$

where D_D represents the D-optical isomer of aspartic acid.

Further sets of catamers were synthesized with the general formulae:-

$$Y_2\text{-}D_5\text{-}Sp\text{-}P_D\text{-}F\text{-}A\text{-}D_D\text{-}Lk\text{-}(solid\ support)$$

and
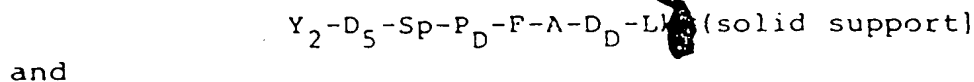
$$Y_1\text{-}P_D\text{-}F\text{-}A\text{-}D_D\text{-}Sp\text{-}D_5\text{-}Lk\text{-}(solid\ support)$$

where the extended set of monomers was used in the designated position D_5. When these sets of catamers were reacted with the monoclonal antibody it was found that the catamer which reacted most strongly with the antibody was:-

$$Y_2\text{-}R_D\text{-}\beta\text{-}P_D\text{-}F\text{-}A\text{-}D_D$$

where β represents β-alanine and had been included in the synthesis of the catamer as a spacer.

Pretreatment of the monoclonal antibody with human chorionic gonadotrophin completely removed the ability of the antibody to react with the catamer $\beta\text{-}R_D\text{-}\beta\text{-}P_D\text{-}F\text{-}A\text{-}D_D$ thus illustrating the specificity of the mimotope.

EXAMPLE 6

This Example illustrates the application of the invention to the detection of a mimotope to a receptor which is not an antigen to which an antibody has been raised. This Example detects mimotopes of a receptor to which a virion binds. In this case the t? system for detecting binding to catamers has to be modified. In this Example, the synthesized catamers ? allowed to react with influenza virus particles (strai? A-Shearwater/1/72). After reaction the catamers are washed to remove unbound virus particles. The presen? of bound virus particles was detected by reacting with polyclonal antibody (S227-1) which had been raised against the haemaglutinin of influenza virus A/Shearwater/1/72. Antibodies which had reacted with the bound virus were detected in the usual way by ELI?

A set of catamers was synthesized with the general formula:-

$$Y_1-D_2-D_1-Lk-(\text{solid support})$$

where the end group, Y_1-, is β-alanyl-β-alanine. The set of monomers which was used in the designated positions D_1 and D_2 consisted of the D- and L- optical isomers of the common alpha amino acids. When the catamers were reacted with a suspension of influenza virions strain A/Shearwater/1/72, particles bound to dipeptides at the designated positions:-

$$A_D-K$$
$$I_D-K$$
$$N-I_D$$

and

$$M_D-I_D$$

where the suffix "D" indicates the D-optical isomer of the indicated amino acid. After removal of the bound virus from the catamers, the catamers were reacted with the polyclonal antibody S227-1 at the same concentration as that used to detect bound virus to ensure that the peaks found were due to binding of virus rather than binding of the polyclonal antibody.

Further sets of catamers were synthesized with the general formulae:-

$$Y_2-D_3-Sp-A_D-K-Lk-(solid\ support)$$
$$Y_1-A_D-K-Sp-D_3-Lk-(solid\ support)$$
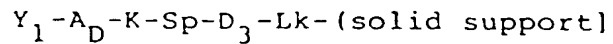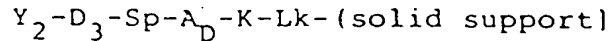
where Y_1- represents the β-alanine end group and Sp is an element of the set of spacers, (null, β-alanine). The set of monomers used at the designated position D_3 was the extended set as described in Example 5. When these sets of catamers were reacted with influenza virus strain A/Shearwater/1/72, virions bound most strongly to the catamer:-

$$Y_1-A_D-K-K_D$$

This Example demonstrates that the invention can be applied to the determination of mimotopes of receptor molecules and ligands in general. Implementation of the method is limited only by the ability to detect the presence of binding to the set of catamers.

CLAIMS:

1.   A method of detecting or determining the sequence of monomers which is a topographical equivalent of the ligand which is complementary to a particular receptor of interest, the method comprising the steps of:-

    1.   synthesizing a plurality of catamers, each said catamer being of the general formula:

$$D_2-D_1$$

        wherein D_1 represents a designated monomer selected from a first set of monomers, and D_2 represents a designated monomer selected from a second set of monomers which may be the same as or different to said first set of monomers; said plurality of catamers comprising catamers in whi h each designated monomer is systematically varied to contain members from the respective set of monomers;

    2.   contacting each catamer with the receptor of interest, and,

    3.   detecting or determining the presence or absence of binding between each catamer and said receptor.

2.   A method according to claim 1, comprising the further steps of:-

    a.   synthesizing a further plurality of catamers, each said catamer being of the general formula:

$$D_3-D_2-D_1, \text{ or}$$
$$D_2-D_1-D_3$$

wherein D_1 and D_2 are as defined above, in claim 1, and D_3 represents a designated monomer selected from a third set of monomers which may be the same as or different to either the first or the second set of monomers, and

b.   performing steps 2 and 3 as defined in claim 1 with said further plurality of catamers.

3.   A method according to claim 2, wherein steps a. and b. are repeated with the systematic addition of further monomers to the catamers.

4.   A method according to any one of claims 1 to 3 comprising the further steps of:-

A.   synthesizing a plurality of additional catamers, each said additional catamer being of the general formula:

$$D_2-Sp-D_1$$
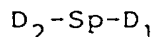
wherein D_1 and D_2 are as defined in claim 1 and Sp is a spacer molecule which can modify the relative orientation of the monomers D_1 and D_2;   and

B.   performing steps 2 and 3 as defined in claim 1 with said plurality of additional catamers.

5.   A method according to claim 2 or claim 3, wherein steps a. and b. are repeated with the systematic introduction of a spacer molecule Sp as defined in claim 4 into all possible positions of the further pluralities of catamers as defined in claim 2 or claim 3.

6.   A method according to any one of claims 1 to 5, comprising the further step of systematically replacing the monomers in any of the catamers referred to above with their optical isomers, either individually or in combinations of monomers, and again performing steps 2 and 3 as defined in claim 1.

7.   A method according to any one of claims 1 to 6, comprising the further step of systematically replacing the monomers in any of the catamers which bind with the receptor of interest, either individually or in combinations of monomers, with other monomers selected from the respective set(s) of monomers, and again performing steps 2 and 3 as defined in claim 1.

8.   A method according to claim 1, wherein each of said plurality of catamers is synthesized on a solid support, and has the general formula:-

$$Y-D_2-D_1-Lk-(solid \; support)$$
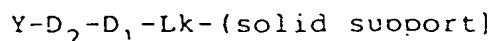
wherein D_1 and D_2 are as defined in claim 1, Lk represents a linker molecule, and Y is an end group of the catamer.

9.   A method according to claim 4, wherein each of said plurality of additional catamers is synthesized on a solid support, and has the general formula:-

$$Y-D_2-Sp-D_1-Lk-(solid \; support)$$
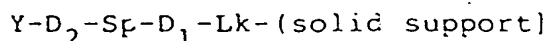
wherein D_1 and D_2 are as defined in claim 1, Sp is as defined in claim 4, and Lk and Y are as defined in claim 8.

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[4] G01N 33/53, 33/68

II. FIELDS SEARCHED

Minimum Documentation Searched

Classification System	Classification Symbols
IPC	G01N 33/16, 33/54, 33/53, 33/68, 33/50
US Cl.	436-501, 435-7

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched

AU : IPC as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	WO,A1, 84/03564 (COMMONWEALTH SERUM LABORATORIES COMMISSION) 13 September 1984 (13.09.84)	1-9
X	AU,A, 25428/84 (COMMONWEALTH SERUM LABORATORIES COMMISSION) 20 September 1984 (20.09.34)	1-9
X	Scientific American, February 1983 pages 48-56 (page 50 lines 33-39), R.A. Lerner, "Synthetic Vaccines"	1-9
P,X	WO,A1, 85/02121 (THE WISTAR INSTITUTE) 23 May 1985 (23.05.85)	1-9
X	WO,A1, 84/02983 (PRIMUS) 2 August 1984 (02.08.84)	1-9
A	AU,B, 73787/84 (476619) (BAXTER LABORATORIES, INC) 1 April 1976 (01.04.76)	1-9
P,X	AU,A, 45339/85 (COMMONWEALTH SERUM LABORATORIES COMMISSION) 30 January 1986 (30.01.86)	1-9
P,X	AU,A, 36563/84 (MILES LABORATORIES, INC) 20 June 1985 (20.06.85)	1-9
P,X	AU,A, 36562/84 (MILES LABORATORIES, INC) 20 June 1985 (20.06.85)	1-9
P,X	AU,A, 36560/84 (MILES LABORATORIES, INC) 20 June 1985 (20.06.85)	1-9

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
27 June 1986 (27.06.86)	17 July 1986 (17-7-86)

International Searching Authority	Signature of Authorized Officer
Australian Patent Office	J.H. CHAN

Form PCT/ISA/210 (second sheet) (January 1945)